Psychological Reports, 1986, 58, 3-9. © Psychological Reports 1986

SEROTONIN, SEPTAL LESIONS, AND SHOCK-ESCAPE LEARNING IN RATS[1]

BRUCE A. MATTINGLY, JAMES E. GOTSICK, AND E. BROOKS APPLEGATE

Morehead State University

Summary.—The involvement of reduced serotonin in deficient leverpress, shock-escape performance of rats with septal lesions was assessed in two studies. In Exp. 1, rats were treated with either para-chlorophenylalanine (PCPA) or saline and then tested in a leverpress, shock-escape task. In Exp. 2, rats with septal lesions and sham-operated control rats were treated daily with either 5-hydroxytryptophan (5HTP) or saline and tested on the same shock-escape task. Primary findings were as follows: (a) rats treated with PCPA learned to escape shock as quickly as saline control rats; (b) the shock-escape performance of rats with septal lesions was significantly inferior to that of control rats; and (c) the administration of 5HTP did not significantly improve the performance of either lesioned or control rats. These results suggest that the reduction of brain serotonin induced by septal lesions is not involved in the deficient shock-escape performance of septal-lesioned rats.

Following damage to the septal nuclei of the limbic forebrain, there is a significant reduction of brain serotonin levels. Smith (1979a) has suggested that this lesion-induced decrease of brain serotonin may be responsible for many of the behavioral effects of septal lesions in aversive learning situations. This view is supported primarily by the similarities observed between the behavioral effects of septal lesions and the effects of para-chlorophenylalanine (PCPA), a drug which depletes brain serotonin. As examples, both septal lesions and PCPA treatments in rats increase pain sensitivity, disrupt passive-avoidance learning, and facilitate two-way active-avoidance learning; see Caplan (1973) and Peters, Anisman, and Pappas (1978) for review. Moreover, injections of 5-hydroxytryptophan (5HTP), a drug which increases brain serotonin levels, reverses the septal-lesion induced facilitation of active avoidance learning (Smith, 1979a).

The objective of the present study was to determine whether lesion-induced reductions in brain serotonin are involved in other behavioral effects of septal lesions in aversive learning tasks. Specifically, the present study investigated the involvement of brain serotonin reductions in the deficient leverpress shock-escape performance of rats with septal lesions (Gotsick, Osborne, Allen, & Hines, 1971).

[1]This research was supported by a Faculty Research Grant from Morehead State University. The authors thank M. Graham, J. Spencer, and J. Vicedomini for their assistance in behavioral testing and histology. Requests for reprints should be sent to Bruce A. Mattingly, Department of Psychology, Morehead State University, Morehead, Kentucky 40351.

EXPERIMENT 1

The purpose of Exp. 1 was to determine the effect of a drug-induced interference with serotonergic functioning on the leverpress, shock-escape performance of normal rats. According to a serotonin-reduction view of septal lesion effects, normal rats treated with PCPA should be deficient in shock escape learning. In Exp. 1, therefore, rats were treated with either PCPA or saline and then tested on a leverpress, shock-escape task.

Method

Subjects.—Twenty-two male Wistar albino rats were experimentally naive and approximately 90 days old at the beginning of testing. All rats were housed individually and maintained on *ad lib.* food and water. A 12-hr. light-dark cycle was held constant throughout the experiment.

Apparatus.—Behavioral testing was conducted in two Grason-Stadler operant conditioning chambers (Model III) housed individually in sound-attenuated research chests. These chambers had grid floors, and a house light (GE 1820) and response lever mounted on one wall. Grason-Stadler constant current shock generators (Model 700) equipped with grid scramblers were used to deliver footshock.

Procedure.—Approximately 72 hours before the beginning of testing, the rats were randomly assigned in equal numbers to either the PCPA or saline group. The PCPA rats received an intraperitoneal (IP) injection of 320 mg/kg of D, L para-chlorophenylalanine methylester hydrochloride and the rest of the rats were injected with an equivalent volume of saline. All doses were calculated as the active base of the drug and dissolved in isotonic saline just prior to administration. Also, all doses were administered in a volume of 2 ml/kg and treatment conditions were coded so that group assignments were unknown to the experimenter during both injection and testing procedures. Following the injection all rats were returned to their home cages.

Shock-escape training was initiated three days following the drug injections. In each training session, the rat was placed in one of the chambers and 90 sec. later, a 1.3-mA footshock was delivered to the gridfloor. This shock continued for 1 min. or until the rat pressed the lever. The shock trials were separated by 90-sec. intertrial intervals. Each daily session consisted of 10 discrete shock-escape trials, and all animals were tested for seven consecutive days. During the sessions, response latencies to the nearest .001 sec. were recorded. Also, the total number of leverpresses per session and the total amount of time the lever was depressed during each session was recorded.

Results

Fig. 1 presents the mean speed scores for the PCPA and saline groups across the seven shock-escape sessions. Speed scores were derived by adding

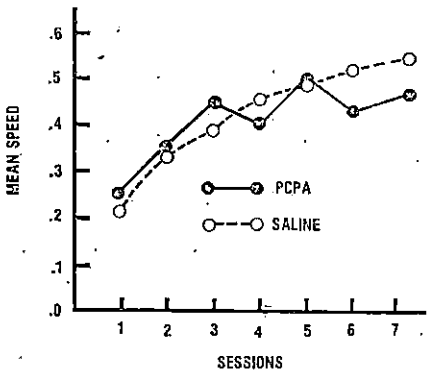

FIG. 1. Mean speed scores across the seven shock-escape sessions for the saline- and PCPA-injected rats

the integer one to each latency and then taking the reciprocal [1/(LAT+1)]. This transformation prevents very short or very long latency scores from making a disproportionate contribution to the mean performance scores (Osborne, 1971). The possible range of transformed scores is from 0 to 1, with larger numerical values representing faster response speeds.

As may be seen in Fig. 1, both groups of rats displayed an increase in speed scores across the seven sessions and there were few differences in performance between the PCPA- and saline-treated rats. An analysis of variance performed on these data showed, as expected, a significant effect for session ($F_{6,120} = 21.65$; $p < .001$) but neither the main effect of drug nor the interaction of drug $\times$ session was significant. Moreover, the analyses of variance performed on the number of leverpresses and the amount of time the lever was depressed per session indicated no significant differences between the PCPA- and saline-treated rats.

Discussion

As mentioned, rats with septal lesions are severely retarded in leverpress-shock learning. It has been suggested that the inferior performance of septally-lesioned rats is based on an inability of these rats to remain near the lever during the intertrial intervals. Whereas normal rats learn to stay near the lever and hold it down during the intertrial intervals, septally-lesioned rats initially spend very little time depressing the lever and in contrast to normal rats, show little or no increase in depressions during the intertrial interval across sessions (Gotsick, *et al.*, 1971).

The results of this experiment indicate that rats treated with a dose of PCPA which has been reported to produce decreases of 80% or more in brain serotonin levels (Koe & Weisman, 1966), learn to escape shock as quickly as saline control rats. Indeed, PCPA-treated rats did not differ significantly from saline rats in the speed of responding, the number of leverpresses, or in the amount of time the lever was depressed.

These differences in shock-escape performance between PCPA-treated rats and septally-lesioned rats suggest that the reduction in brain serotonin which accompanies septal lesions is not an important factor in the inferior shock-escape performance of septally-lesioned rats. Moreover, since both PCPA and septal lesions increase sensitivity to footshock (Smith, 1979b), the present findings suggest that altered sensitivity to footshock is not the critical determinant of the retarded shock escape performance in rats with septal damage.

EXPERIMENT 2

Although PCPA injections did not affect shock escape learning, it is still possible that serotonin reductions may be important in producing the deficient shock-escape performance of rats with septal lesions. For instance, injections of PCPA result in over an 80% decrease in serotonin levels in widespread areas of the forebrain (Koe & Weisman, 1966). In contrast, septal lesions result in only a 20 to 30% decrease in serotonin levels, and this decrease is found primarily in the hippocampus and cortex (Gage, Thompson, & Valdes, 1978; Smith, 1979b). This difference in the extent of depletion of serotonin might account for differences in shock-escape performance between septally-lesioned and PCPA-treated rats.

Smith (1979a, 1979b) has reported that doses of 105 mg/kg of 5-hydroxytryptophan (5HTP) return levels of serotonin to near normal in septally-lesioned rats and attenuate the lesion-induced facilitation of active avoidance learning. The purpose of Exp. 2, therefore, was to determine the effect of 5HTP on the shock-escape performance of septally-lesioned and sham-operated control rats. If reduced serotonin levels are involved in the deficient shock-escape performance of septally-lesioned rats, then administration of 5HTP should produce a significant improvement in the lesioned animals' performance.

Method

Forty male Wistar albino rats were experimentally naive and approximately 100 days old at the beginning of testing. Rearing and behavioral testing procedures were the same as in Exp. 1. Fourteen days prior to shock-escape testing, 20 randomly assigned rats received septal lesions and the remainder were given sham operations. All surgery was performed under ether anesthesia. Bilateral lesions were produced through a stereotaxically oriented electrode connected to a Radionics radio frequency lesion maker (Model RFG-4). The electrode tip was positioned 1.8 mm anterior to Bregma, .5 mm lateral to the midline at an angle of 5°, and 5.0 mm below dura. Current was passed for 20 sec. and the tip temperature reached 65-70°C. Rats in the control group were anesthetized and placed in the Stoelting stereotaxic instrument (Model 51200); the scalp was incised but the skull was not penetrated. At the con-

clusion of behavioral testing, all lesioned animals were sacrificed with ether anesthesia and perfused intracardially with physiological saline followed by a 10% formalin solution. The brains were then removed and after sufficient fixation in 10% formalin, frozen coronal sections were cut at 50 μ and stained with cresyl violet.

Following surgery, all septal animals displayed the hyperreactivity normally associated with the lesion (Gorsick & Marshall, 1972). Consequently, both groups of rats were handled for approximately 5 min. daily until the beginning of behavioral testing. At the beginning of shock escape testing, septal and control rats were equally docile.

Shock-escape training was the same as in Exp. 1 except the animals were tested for eight consecutive days. Also, one-half the rats in each group received an IP injection of 105 mg/kg D,L-5-Hydroxytryptophan daily 30 min. prior to testing and the rest of the rats received an equal volume of saline. In summary, a 2 (septal vs sham-lesion) $\times$ 2 (5-HTP vs saline) factorial design with repeated measures was used.

Results

Anatomical.—Examination of brain sections showed large bilateral lesions of the septum in all experimental animals. The typical lesion extended anteriorly to the nucleus accumbens, posteriorly to the columns of the fornix, dorsally to the corpus callosum, and ventrally to the anterior commissure. Laterally, the lesions were bounded by the caudate nucleus. In most animals, the nucleus accumbens, columns of the fornix, and corpus callosum received minor damage.

Behavioral.—Fig. 2 presents the mean speed scores for the four groups across the eight shock-escape sessions. Speed scores were derived as in Exp. 1. As may be seen, the sham-operated control rats responded more quickly than the lesioned rats ($F_{1,36} = 34.30$, $p < .001$). Further, the speed scores increased across the daily sessions ($F_{7,252} = 26.69$, $p < .001$), but this increase

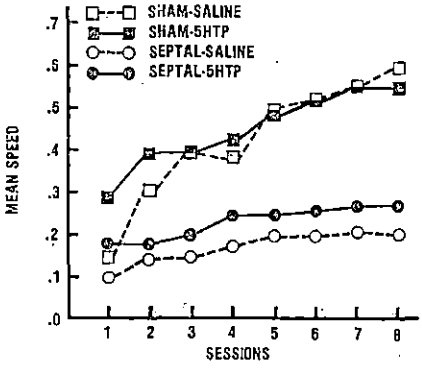

FIG. 2. Mean speed scores across the eight shock-escape sessions for rats with septal lesions and sham-operated control rats injected with either saline or 5-HTP

was much greater for the control rats than for the lesioned rats ($F_{7,252} = 7.54$, $p < .001$). Although 5-HTP appeared to increase slightly the speed scores of the lesioned animals, neither the main effects of drug nor any of the interactions including drug as a factor were significant.

Consistent with the speed data, the analyses performed on the number of leverpresses and the amount of time the lever was depressed per session did not indicate any reliable effects of drug. These analyses did, however, show significant effects of lesion as the lesioned rats, relative to the sham animals, made fewer leverpresses ($F_{1,36} = 21.37$, $p < .001$) and spent less time depressing the lever ($F_{1,36} = 35.66$, $p < .001$).

Discussion

Manipulations which decrease brain serotonin, such as septal lesions and PCPA treatments, facilitate active avoidance learning (Peters, *et al.*, 1978; Smith, 1979a). In addition, this facilitation can be reversed by the administration of 5HTP to septally-lesioned rats (Smith, 1979a). These findings support the view that the reduced serotonin levels produced by septal lesions are involved in the facilitated active avoidance performance of these rats.

The results of the present experiment indicate that septal lesions significantly retard leverpress, shock-escape performance in rats. This finding, of course, is consistent with previous studies (Gotsick, *et al.*, 1971). In contrast to the avoidance-learning studies however, the present results indicate that administering 105 mg/kg of 5HTP daily to septally-lesioned rats does not significantly affect their shock-escape performance. These results are in agreement with those of Exp. 1, which indicated that depletion of brain serotonin in normal rats does not significantly affect leverpress, shock-escape performance. Taken together, the results of these two experiments suggest that the reduction of brain serotonin which accompanies lesions of the septum does not retard shock-escape performance in rats with this lesion.

It is, of course, possible that neither PCPA nor 5HTP affected shock-escape learning in the present study because the drugs did not have the intended effect on serotonin levels. That is, although the neurochemical effects of both drugs have been well documented (Peters, *et al.*, 1978), errors in storage, mixing, or injecting the drugs could have decreased their effectiveness. Although without a neurochemical analysis on the animals tested this possibility cannot be ruled out with certainty, there are several arguments against this view. First, using the same storage, mixing, and injection techniques we have found significant behavioral effects using PCPA (Mattingly, Chandler, Applegate, & Brunelle, 1984). Likewise, we have informally observed "headshake" responses in normal rats following IP injections of 5HTP. This "headshake" response to 5HTP has been suggested to be directly related to serotonin-receptor stimulation (Colpaert & Janssen, 1983). It seems likely, therefore, that the drugs used had the expected effects on serotonin levels.

Besides serotonin, septal lesions also produce a reduction in the levels of other putative neurotransmitters. For example, acetylcholine levels are reduced following septal lesions and, interestingly, many similarities exist between the behavioral effects of cholinergic receptor blockade and septal lesions (Fried, 1972). Moreover, we have recently found that the cholinergic antagonist, scopolamine, but not methylscopolamine, produces a disruption in leverpress, shock-escape performance in rats similar to that produced by septal lesions (Mattingly, 1985). It is probable, therefore, that the various effects of septal lesions in aversive-learning situations are mediated by different behavioral and biochemical mechanisms.

REFERENCES

CAPLAN, M. (1973) An analysis of the effects of septal lesions on negatively reinforced behavior. *Behavioral Biology*, 9, 127-167.

COLPAERT, F. C., & JANSSEN, P. A. J. (1983) The head-twitch response to intraperitoneal injection of 5-hydroxytryptophan in the rat: antagonist effects of purported 5-hydroxtryptophan antagonists and of pirenperone, an LSD antagonist. *Neuropharmacology*, 22, 993-1000.

FRIED, P. A. (1972) Septum and behavior: a review. *Psychological Bulletin*, 78, 292-310.

GAGE, F. H., THOMPSON, R. G., & VALDES, J. J. (1978) Endogenous norepinephrine and serotonin within the hippocampal formation during the development and recovery from septal hyperreactivity. *Pharmacology, Biochemistry, & Behavior*, 9, 359-367.

GOTSICK, J. E., & MARSHALL, R. C. (1972) Time course of the septal rage syndrome. *Physiology & Behavior*, 9, 685-687.

GOTSICK, J. E., OSBORNE, F. H., ALLEN, C. J., & HINES, K. M. (1971) Factors affecting performance on a shock escape task in rats with septal lesions. *Physiology & Behavior*, 6, 199-201.

KOE, K. B., & WEISMAN, A. (1966) P-chlorophenylalanine: a specific depletor of brain serotonin. *Journal of Pharmacology and Experimental Therapeutics*, 154, 499-516.

MATTINGLY, B. A. (1985) Scopolamine disrupts leverpress shock escape learning in rats. Manuscript submitted for publication.

MATTINGLY, B. A., CHANDLER, H., APPLEGATE, E. B., & BRUNELLE, M. (1984) Activity during a conditioned aversive stimulus in rats treated with para-chlorophenylalanine. *Psychological Record*, 34, 389-396.

OSBORNE, F. H. (1971) Varied reward and simple escape. *Psychological Reports*, 29, 1231-1236.

PETERS, D. A. V., ANISMAN, A., & PAPPA:, B. A. (1978) Monoamines and aversively motivated behavior. In H. Anisman & G. Bignami (Eds.), *Psychopharmacology of aversively motivated behavior*. New York: Plenum.

SMITH, R. F. (1979) Attenuation of septal lesion-induced shuttlebox facilitation by 5-hydroxytryptophan. *Physiological Psychology*, 7, 419-421. (a)

SMITH, R. F. (1979) Mediation of footshock sensitivity by serotonergic projection to hippocampus. *Pharmacology, Biochemistry, & Behavior*, 10, 381-388. (b)

Accepted November 4, 1985.